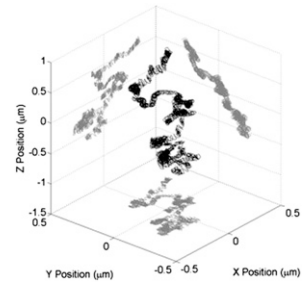
Cramer-Rao lower bound-limited single molecule localization with an SCMOS camera. Combining this with a recently developed multi-emitter fitting algorithm and optimized imaging condition, we show that this technique shortens the typical acquisition time for fixed samples by up to two orders of magnitude without compromising the field of view. Furthermore, we demonstrate video-rate super-resolution microscopy of live cells by monitoring the transient clustering events of transferrin receptors with a reconstructed frame rate of 32 fps.
The here presented method allows to replace EMCCD cameras with SCMOS technology and record faster and more precise super-resolution images without compromises.

2695-Pos Board B714
Effects of Photoactivation Efficiency on Single Molecule Counting in PALM
Nela Durisic, Lara Laparra-Cuervo, Angel Sandoval, Melike Lakadamyali.
ICFO – Institute of Photonic Sciences, Castelldefels (Barcelona), Spain.
We combine single molecule photobleaching with super-resolution fluorescence imaging to count the absolute number of proteins in small oligomers using PALM.
First we employed single molecule imaging and stepwise photobleaching of fluorescently tagged glycine-gated ion channels (GlyR), to determine subunit stoichiometry of this neurotransmitter to be 3α1:2β (Durisic et. al JNeuroSci 2012). Then the channel was used as a template to precisely characterize photoactivation and photoconversion efficiency at the single molecule level of most commonly used fluorescent proteins in PALM microscopy. using stepwise photobleaching we characterized the effects of dense sample labeling and determined that only a subset of fluorescent proteins can be efficiently photoacivated or photoconverted. We could then directly relate these results to the number of GlyR subunits per channel counted by means of PALM microscopy. Our findings have important implications for super-resolution microscopy, particularly in the context of quantitative imaging and co-localization in multi-color super-resolution experiments.

2696-Pos Board B715
Revealing the Stoichiometry of G Protein-Coupled Receptors (GPCRs) at the Cell Surface using Single Molecule Imaging
Laura Weimann[1], Steven F. Lee[1], James H. Felce[2], Simon J. Davis[2], David Klenerman[1].
[1]Department of Chemistry, University of Cambridge, Cambridge, United Kingdom, [2]Nuffield Department of Clinical Medicine and MRC Human Immunology Unit, University of Oxford, John Radcliffe Hospital, Headington, Oxford, United Kingdom.
The human genome contains more than 800 G protein-coupled receptors (GPCRs); overall, 3-4% of the mammalian genome encodes these molecules. Processes controlled by GPCRs include neurotransmission, cellular metabolism, secretion, and immune responses. At present their functional mechanism remains controversial and poorly understood: it is believed however that the stoichiometry of these receptors is strongly correlated with their function. Initially GPCR receptors were thought to be monomeric. Several studies favor the concept that GPCR form dimers and are not capable of signaling as independent monomers. Recent single-molecule fluorescence studies have attempted to solve this dilemma by suggesting that GPCRs form transient dimers with a lifetime of ~100 ms. Yet questions remain regarding the physiological relevance of these studies, since they have not been performed at physiological receptor densities.
Therefore we label individual GPCRs in HEK 293T cells at varying expression levels using HaloTag-coupled dyes and record their fluorescence by single molecule total internal reflection fluorescence (smTIRF) microscopy. We are then able to determine receptor stoichiometry dependent on receptor density by direct observation and quantitative counting of the number of photobleaching steps within a single receptor. After testing the method with receptors of known stoichiometry, we apply it to study the oligomerisation state of the β_2 adrenoceptor, the chemokine receptor CCR5 and the muscarinic receptor M1.

2697-Pos Board B716
Three-Dimensional Tracking of Fluorescent Particles in Confocal Microscopy
Trevor Ashley, Catherine Chan-Tse, Jeffrey Worthey, **Sean Andersson**.
Boston University, Boston, MA, USA.
Three-dimensional tracking of fluorescent particles has the potential of offering scientists much more information pertaining to biological phenomena than can be provided with two-dimensional tracking. In this work, we describe a tracking algorithm that can be used in any optical configuration where a two- or three-dimensional point spread function is sampled in space and time. We show preliminary results from the application of this algorithm to three-dimensional tracking of freely-diffusing fluorescent quantum dots in a confocal microscope. Further, using experimental data, we illustrate how the algorithm can maintain tracking in low-SNR settings where high-background noise may be present or where low-excitation power is required due to concerns of phototoxicity. We describe the versatility of the algorithm in tracking fluorescent particles of various diffusion coefficients and velocities by the adjustment of tuneable parameters. Since the algorithm does not estimate the position of the particle, the user may choose any localization algorithm on the resulting trajectories. In our analysis, we use a GPS-like localization technique called fluoroBancroft to estimate the particle position from the resulting trajectories and describe results from Monte Carlo simulations that indicate optimal window sizes.



2698-Pos Board B717
Reconstruction of Electron Densities from Few Photon Singular Molecule X-Ray Scattering Experiments
Benjamin von Ardenne.
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.
X-ray free electron lasers in single molecule experiments hold the promise of solving macromolecular structures. To that aim, reliable structure reconstruction from noisy scattering images with rigorous bounds for the statistical uncertainty is required, which in fact represents one of the main challenges in the field. I present an approach that uses the photon correlations of single x-ray shots to reconstruct the original electron density. using the spherical harmonics expansion of the Fourier transformation of the electron density, an analytic expression of the two and three photon correlation is derived. We demonstrate that structural information can be derived from the correlations and estimate the achievable resolution.

2699-Pos Board B718
Molecular Transport across the Hetero-Oligomeric Cell Wall Porins Derived from Gram Positive Bacterium Nocardia Farcinica
Pratik R. Singh, Iván Bárcena-Uribarri, Roland Benz, Kozhinjampara R. Mahendran, Mathias Winterhalter.
Jacobs University Bremen, Bremen, Germany.
Cell needs to perform selective and controlled transport of vital ingredients to and from the cell to ensure its viability. These processes are regulated by various protein channels present in the cell membrane. For example, Gram-negative bacteria contains channels of nanometer dimensions that are either specific to a certain molecule, eg. Maltoporin to maltose, or allow a wide range of molecules to pass through the them, eg. OmpF. In contrast, Gram-positive bacteria do not have large pore forming proteins in their outer membrane. However, it has been studied that the class of bacteria such as Mycobateria, Corynebacteria, Nocardia belonging to the order of actinomycetales from Gram-positive bacteria have channel forming proteins in their outer membrane mycolic acid layer. In this work we characterize the transport of macromolecules across the cell wall porins of Nocardia farcinica, known to be a dangerous pathogen causing Nocardiosis. An adequate method to study properties of these channels is electrophysiology and in particular analyzing the ion current fluctuation in the presence of permeating solutes provides information on possible interactions with the channel surface. The translocation of small solutes, such as sugars, peptides and antibiotics, through the channel has been studied by ion current fluctuation analysis at single molecular level.

2700-Pos Board B719
Applying Single Molecule Fluorescence Microscopy to Precisely Count Absolute Numbers of Proteins in Living Cells
Matthieu G.S. Palayret[1], Steven F. Lee[1], Ricardo A. Fernandes[2], Helena Browne[1], Simon J. Davis[2], David Klenerman[1].
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Oxford, Oxford, United Kingdom.
Counting absolute numbers of proteins is of major importance in biology. This would help for example to resolve the stoechiometry of complexes, to observe aggregation phenomena or to describe the minimal signal required for a specific cellular response. The only existing tools available to biologists are bulk experiments, crystallography or photobleaching steps. Each of these methods comes with its own drawbacks: by comparing global mean values, bulk experiments do not take into account quenching or differences of brightness in a population of fluorophores; crystallography only studies the protein as a crystal and hence in a totally artificial environment; finally, the detection of photobleaching steps